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Claims 1-6 and 18-20 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claims 1-6 and 18-20 are rejected over the recitation of the phrase "cloning the **on** or more genes" in step C) of claim 1. It is not clear if a new gene named "**on**" (which does not have a basis in the Specification or claim) is claimed or "one" is claimed or both are claimed. Applicants have amended claim 1 so that "on" now states "one"

Claim 1 has been amended following a telephone conference with the Examiner on April 23, 2003 for purposes of further distinctly describing the invention. No new matter has been added. The term "flanking" may be found on page 22 of the Application.

### **Claim Rejections - 35 U.S.C. §102 and 35 U.S.C. §103**

Claims 1 and 5-6 have been rejected under 35 U.S.C. §102(b) as being anticipated by Silver, et al. (U.S. Patent No. 4,994,370) (February 19, 1991) and claims 2-4 and 18-20 have been rejected as obvious in light of Silver in combination with secondary references.

Applicants assert that Silver describes a DNA amplification technique which is substantially different from the method of cloning

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one or more genes in a cassette array claimed by the Applicants. In particular, Silver does not anticipate the present claimed invention because the reference does not suggest or teach "each and every element set forth in the claim...either expressly or inherently" (MPEP 2131).

1. Applicants claimed invention relies on known flanking sequences. Silver's method relies on unknown flanking regions.

Claim 1 of the above application is a method for cloning one or more genes in a cassette array where the identified flanking sequences enable the cloning of genes therebetween. Claim 1 has been amended to further emphasize this feature.

In contrast, the Silver reference describes amplifying flanking sequences which are unknown. Silver states in Column 2, line 37 that it is desired to amplify unknown flanking sequences. The alternate strategy in Figure 4 of Silver is to amplify flanking DNA by circularizing single stranded DNA. As an example, Silver describes the use of the known sequence an integrated mouse ecotropic proviral DNA for amplifying unknown flanking sequences of cell DNA.

In the Examiners' response to arguments, some confusion arose concerning the argument on page 6 of the Applicant's response date 12/5/02. Applicants stated that the Silver reference (which is directed to analyzing an unknown cell DNA environment for a murine

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provirus) does not suggest the cloning of genes in a cassette array using flanking repetitive sequences as hybridization targets for primers required in claim 1. In other words-Silver has developed a method for cloning to solve a problem of an unknown DNA environment. This problem is not applicable to claim 1 because the environment of the genes is known (page 15 of the Application).

2. Applicants claimed invention relies on repetitive sequences in the known flanking regions. Silver does not suggest or teach repetitive sequences.

Claim 1 of the above application requires hybridizing primers to repeat sequences (step (a)).

In contrast, the Silver reference describes oligonucleotide primers that do not hybridize to any repeat sequences. The dinucleotide (GG) in Figure 4 is not a repeat sequence but merely a duplicated single nucleotide. Moreover, this dinucleotide is not the target of oligonucleotide primers.

3. Applicants claimed method requires hybridizing primers to repeat sequences for amplification of sequences therebetween followed by ligation of the fragments into a vector for cloning. Silver teaches ligation of the DNA prior to amplification.

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Claim 1 of the above Application describe steps that must follow in order. The order has been further emphasized by adding reference to previous steps in each element. This order requires that amplification of DNA precedes ligation.

In contrast, the Silver reference describes a ligation step prior to amplification.

In summary, the Silver reference is substantially different from the claimed invention described in claim 1 and consequently dependent claims 5 and 6 for the above reasons. Consequently, the rejections based on the combination of the Silver reference with the secondary cited references of Marshall, Xu, Stein, Gruber and Coruzzi for dependent claims 2-4 and 18-20 are moot.

For the reasons set forth above, Applicants respectfully request that the rejections set forth in the Official Action of December 27, 2002 be withdrawn and submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the remarks made herein, the undersigned attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

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Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

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**Claims now pending**

1. (amended) A method for cloning one or more genes in a cassette array, the array being characterized by a plurality of genes where each gene is embedded in a predictable nucleotide sequence context including a repeat DNA sequence, the method comprising the steps of:

- (a) [identifying the repeat sequence in the cassette array (b)] hybridizing oligonucleotide primers to identified flanking repeat sequences in the cassette array; [and]
- (b) amplifying the DNA between the hybridized primers of step (a) to produce DNA fragments which contain one or more genes; and
- (c) ligating the DNA fragments of step (b) into a vector for cloning the [on] one or more genes in a host cell.

2. The method of claim 1 wherein the one or more genes are selected from the group of peptides consisting of: adhesins, pilus proteins and outer membrane proteins; transporter peptides; toxins; hemolysins; hemagglutins; signaling peptides; detoxifying enzymes; catabolic enzymes specific for compounds episodically available, excluding compounds in the tricarboxylic acid cycle; and enzymes for biosynthesis of rare sugars, excluding ribose, deoxyribose; and sugars of the cell wall and the pericellular envelope.

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3. The method of claim 2 wherein said diversity-selected genes comprise restriction endonuclease genes.

4. The method of claim 2 wherein said diversity-selected genes comprise methyltransferase genes.

5. The method of claim 1 wherein said oligonucleotides contain recognition sites which permit directional cloning.

6. The method of claim 5 wherein the DNA fragments are ligated into said vector in an orientation that enables expression.

7. A method for identifying the presence of gene cassette arrays from within a target DNA preparation, said method comprising the steps of:

(a) hybridizing at least one oligonucleotide which hybridizes to one or more of SEQ ID NO:5 through SEQ ID NO:78 to a DNA preparation; and

(b) detecting the presence of a stable DNA-DNA hybrid.

8. The method of claim 7 wherein said detection comprises determining the presence of stable DNA-DNA hybrid by Southern blot or dot blot.

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9. The method of claim 7 wherein said detection comprises employing at least two oligonucleotides and hybridizing said oligonucleotides to said DNA preparation, and detecting their ability to support DNA polymerization at the 3' end of the stable DNA-DNA hybrid.

10. The method of claim 7 wherein said oligonucleotides comprise SEQ ID NO:79 through SEQ ID NO:91.

11. The method of claim 7 wherein said oligonucleotides hybridize to one or more of DNA SEQ ID NO:5 through SEQ ID NO:78 or portions thereof.

12. The method of claim 7 wherein the DNA source comprises an individual strain.

13. The method of claim 7 wherein the DNA source comprises a group or pool of strains.

14. The method of claim 7 wherein the DNA source comprises environmental DNA.

15. A composition consisting of isolated DNA primers comprising SEQ ID NO:79 through SEQ ID NO:91 or portions thereof.

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16. A composition consisting of DNA primers which hybridize to one or more of DNA SEQ ID NO:5 through SEQ ID NO:78 or portions thereof.

17. A method for identifying gene cassette arrays from a predetermined DNA sequence, said method comprising the steps of:

- (a) screening the said predetermined DNA sequence for TAACWA;
- (b) screening the said predetermined DNA sequence for CGTTRR;
- (c) screening for DNA segments wherein the 5' T of step A is less than about 200 base pairs form the 3' R of step B; and
- (d) determining whether the DNA sequence of step C is repeated in the predetermined DNA sequence.

18. The method of claim 2, wherein the adhesins are fimbrial proteins.

19. The method according to claim 2, wherein the signaling peptides are kinases.

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20. The method according to claim 2, wherein the detoxifying enzymes are drug resistance determinants.